STUDIES OF DIAPAUSE IN THE PINK BOLLWORM IN PUERTO RICO

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INTRODUCTION

The diapause, or resting stage, of the pink bollworm, (Pectinophora gossypiella Saund.), has been studied under many different environmental conditions, yet there is still much disagreement as to the factors which initiate its development and limit its duration. Puerto Rico, a semitropical country, is an ideal place for the study of diapause phenomena since low temperature is not encountered. The resting stage of the pink bollworm is a true diapause, since development in a small number of cases is arrested spontaneously even under apparently favorable climatic conditions.

Larval behavior of this pest has been studied in relation to definite changes in the environmental complex over a period of years in order to determine the factors responsible for the development and duration of the diapause in the field. In the laboratory controlled experiments were conducted in which environmental changes were induced in order to aid in the interpretation of the effect of the environmental complex on the larval stage.

MOISTURE AS A FACTOR INDUCING THE DIAPAUSE

Effects of Rainfall on Abundance of Larvae Going through the Diapause

The abundance in Puerto Rico of the resting stage in the field was determined in four localities over a period of two years, and these records have been correlated with rainfall in order to show their relationship. These data are shown in table 1. It will be noted that larvae going through the diapause occurred in abundance only during periods of drought, i.e., 2 inches or less of rainfall 30 days prior to the date of observation or less than 5.5 inches 45 days prior. This relationship was most clearly shown during the period 1935 at Isabela. An abundance of resting larvae occurred during January, February, and March, a period of severe drought. Very few long-cycle larvae occurred from June to December 1936, a period of heavy rainfall. During another period of drought, November 1937 to January 1938, larvae in the resting stage were again abundant.

From these studies one must conclude that pink bollworms undergoing the diapause occur in abundance during periods of drought regardless of the age of the crop or the time of year at which this takes place. Also a few resting larvae occur regardless of the amount of rainfall, an indication of the existence of an inherited cycle that cannot be broken within several generations.

Table 1.---Relation between rainfall in the preceding 30 and 45 days to changes in abundance of resting larvae of the pink bollworm in green and open cotton bolls. Puerto Rico, 1936 to 1938.

Locality	Type of fruiting form	Date of installation	Number of records	Percentage of resting larvae	Total amount of precipitation during various periods prior to date of installation	
					30 days	45 days
		1936				
Isabela	Open boll	Jan. 8	60	92	1.46	1.46
"	"	" 10	716	65	1.46	1.46
"	"	June 15	271	1	16.21	23.33
"	"	July 14	108	1	5.60	12.71
"	"	" 20	452	2	4.61	11.45
"	"	Aug. 11	270	1	7.30	8.64
"	"	Sept. 4	553	0	9.47	13.50
Boqueron	"	June 23	471	5	3.30	10.60
"	"	Aug. 1	374	25	1.10	1.10
		1937				
Isabela	Green bolls	Sept.15	200	1	2.25	7.14
"	"	" 30	146	8	4.46	6.16
"	"	Nov. 9	114	26	2.19	3.69
Aguadilla	"	Aug. 19	472	0	7.41	8.93
Boqueron	Open boll	Mar. 26	151	89	0	0
"	"	Apr. 17	389	100	1.30	1.30
Mayaguez	Green bolls	Oct. 15	128	0	10.19	11.95
"	"	Nov. 23	200	4	8.29	11.19
Hatillo	"	" 10	71	37	4.23	4.85
		1938				
Isabela	Green bolls	Jan. 10	297	46	4.83	5.55
Mayaguez	"	" 10	305	70	1.32	1.36

Reaction of Larvae to Artificial Drought Conditions in the Laboratory

Methods

Field observations showed that a high percentage of the larvae develop into a resting stage on plants suffering from water deficiency. In order to confirm these field observations, the behavior of pink bollworm larvae developing on plants receiving an abundant supply of water and on plants suffering from water deficiency in a greenhouse was studied.

Cotton plants were grown in 5-gallon oil cans filled with sandy loam soil, to which fertilizer had been added. About two inches of gravel was previously placed on the bottom of each can, and five holes were cut in the bottom to permit drainage. The cans were then placed on boards, 2 feet x 2 feet x 16 inches, on a large platform in the greenhouse. After many bolls had set, 85 days after planting date, the amount of water supplied to 132 plants was so regulated as to cause severe wilting and shedding of leaves and fruiting forms. The remaining plants were given daily applications of water. These plants were vigorous and healthy, bearing many fruiting forms per plant. Blooms on all plants were tagged daily so that the age of the bolls would be known. First-instar larvae were artificially installed on green bolls ranging in age from 11 to 40 days. From 16 to 23 days after date of installation, the larvae were removed from the bolls and classified as to the conditions under which they had developed. The larvae were permitted to spin up between the bottom surface of petri dishes and thin disks of absorbent cotton. This technique permitted observation without disturbing the larvae.

Continuous temperature and relative-humidity records were taken in the insectary and greenhouse by the use of hygrothermographs. The greenhouse was covered on top with transparent glass while the sides were enclosed with fine mesh wire. The mean daily temperature in the greenhouse was 82.4°F., this being 4.4°F. higher than in the insectary. The mean daily relative humidity in the greenhouse was 83.3 percent, this being 3.7 percent higher than in the insectary.

Relation Between the Age of Cotton Bolls and Moisture Content of the Seed and Lint

The moisture content of the seed and lint (the insect's food) of green and open bolls of various ages was determined on October 23rd, and again on November 12th. This was accomplished by drying the material in an oven at 215°F. for 48 hours. As shown in table 2, in all cases except one the moisture content of the seed and lint in the normally watered series was always higher than in the water-stressed

series, this difference becoming greater as the age of the boll increases. The moisture content of the seed and lint was inversely proportional to the age of the boll, regardless of whether the plants suffered from water deficiency or not. The coefficient of correlation between these two factors on October 23 in the normally watered series was -0.986±0.005.

Table 2.---Relation between the age of cotton bolls grown on water-stressed and not water-stressed plants in the greenhouse and the moisture content of the seed and lint on October 23 and November 12, Mayaguez, P. R., 1937.

Stage of growth of the bolls	Age of bolls	Moisture content of the seed and lint			
		Plants not water-stressed		Plants water-stressed	
		Oct. 23	Nov. 12	Oct. 23	Nov. 12
	Days	Percent	Percent	Percent	Percent
Green	27	79.9		77.7	
"	31	74.7		75.8	
"	35	70.2	76.6	69.6	
"	40	64.6		61.4	
"	41	63.8		56.9	
"	42	60.6		52.7	
"	43	63.4		53.4	
"	44	61.8	63.1	53.0	46.4
"	47		57.9		39.4
"	51		53.3		36.9
Open	44		36.7		
"	47		33.8		
"	51		28.4		18.3
"	56				26.0

Relation Between the Moisture Content of the Seed and Lint and Abundance of Larvae in the Diapause

The relation between the moisture content of the seed and lint and abundance of larvae in the diapause was also determined. As shown in table 3, the percentage of resting larvae from bolls on water-stressed plants was always higher than the percentage of long-cycle larvae from bolls grown on the normally watered plants, the averages being 86.4 percent and 57.1 percent, respectively. The moisture content of the seed and lint was inversely proportional to the percentage of resting larvae, the coefficient of correlation being -0.55240.169.

Table 3.----Relation between the percentage of resting pink bollworm larvae, moisture content of the seed and lint, and age of cotton bolls grown on normally watered and water-stressed plants in a greenhouse, Mayaguez, P.R., 1937.

Stage of growth of the bolls	Age of Bolls	Plants not water-stressed: Records	Resting Larvae	Moisture content of seed and lint	Plants water-stressed: Records	Resting Larvae	Moisture content of seed and lint
	Days	Number	Percent	Percent	Number	Percent	Percent
Green		106	41.5	73.4 [1]/			
"	35	167	50.3	76.6	68	79.4 [1]/	62.9 [1]/
"	44	144	49.3	63.1	44	97.7	46.4
"	47	146	71.2	57.9	66	90.9	39.4
"	51	54	72.2	53.3	47	85.0	36.9
Open	44	108	42.6	36.7			
"	47	157	65.0	33.8			
"	51	226	59.7	28.4	103	80.6	18.3
"	56	55	61.8		37	86.5	26.0
Total or Average		1163	57.1	52.9	365	86.4	38.3

[1]/ Age of bolls not definitely known but around 35 days.

Relation Between Various Types of Fruiting Forms and Abundance of Larvae in the Diapause

Since various types of fruiting forms on the cotton plant vary greatly in their moisture content, the percentage of resting larvae that develop in each type was determined. Four types of fruiting forms, namely, young green bolls (soft), old green bolls (hard), young dry bolls (shed when very young, but did not drop from the plant), and open bolls were collected at random in the same field during periods of drought and heavy rainfall. The percentage of resting larvae in each type of fruiting form was determined by the petri dish method as described previously.

As shown in table 4, the highest percentage of long-cycle larvae occurred in the older fruiting forms, i.e., in bolls containing the least amount of moisture.

Table 4.---Relation between the percentage of resting pink bollworm larvae and various types of cotton bolls during a period of drouth and heavy rainfall, Mayaguez, P.R.

Type of fruiting form	Precipitation			
	Heavy 1/		Light 2/	
	Larvae Recorded	Resting Larvae	Larvae Recorded	Resting Larvae
	Number	Percent	Number	Percent
Young green bolls (soft)			95	66.3
Old green bolls (hard)	200	3.5	412	75.7
Young dry bolls 3/	30	3.3	195	79.5
Open bolls	123	20.3	340	92.4

1/ Observations made on Nov. 23, 1937; rainfall Oct., 10.32 in.; Nov. 1 to 23, 7.33 inches.

2/ Observations made on Jan. 20, 1938; rainfall Dec. 0.5 in.; Jan. 1 to 20, 1.6 inches.

3/ Bolls were shed when very young but did not drop off the plant.

EFFECTS OF TEMPERATURE AND MOISTURE ACTING TOGETHER ON THE DEVELOPMENT OF THE DIAPAUSE

In order to determine the effects of temperature and moisture acting together in causing the development of the diapause, numerous fourth-instar short-cycle larvae were placed in desiccators where relative humidities of 1 to 100 percent were maintained at various high temperatures. As checks, larvae were held at room temperature and room relative humidity. The percentage of resting larvae was based on the relative numbers of larvae and pupae 30 days after date of installation.

Three experiments were conducted, and the results are shown in table 5. It will be noted that the highest percentage of resting larvae was found in treatments at 75 percent relative humidity or lower, this being especially true at the higher temperatures. Very few long-cycle larvae were found in treatments maintained at 90 or 100 percent relative humidity regardless of the temperature.

These results again show the importance of moisture in the ecology of this species. Low humidity and high temperature reduce the water content of the larvae by evaporation, thereby decreasing the rate of metabolism and causing cessation of development.

Table 5.---Effects of temperature and moisture on the development of fourth-instar pink bollworm larvae extracted from green cotton bolls, Mayaguez, P.R., 1937.

Treatment	Experiment No. 1				Experiment No. 2				Experiment No. 3			
	80° F. Room temp. [1]		104° F. [2]		78° F. Room temp. [1]		98° F. [3]		77° F. Room temp. [1]		95° F. [4]	
	Larvae Recorded	Resting larvae	Larvae Recorded	Resting larvae	Larvae Recorded	Resting larvae	Larvae Recorded	Resting larvae	Larvae Recorded	Resting larvae	Larvae Recorded	Resting larvae
	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent	Number	Percent
Room R.H. [5]	122	0			146	8.2			114	26.3		
Ca. Cl_2					140	16.4	66	47.0	143	33.6	96	57.3
1% R.H.	178	0	179	96.1	153	13.7	57	54.4	44	11.4	90	45.6
75% R.H.	172	0	168	94.6	129	17.1	69	29.0				
90% R.H.							78	0	130	17.7	87	1.1
100% R.H.					145	0	76	1.3				

[1] Range 69° to 90° F. [2] Range 97° to 113° F. [3] Range 92° to 104° F.

[4] Range 90° to 100° F. [5] Check Average 83% R.H., range 56% to 96% R.H.

MOISTURE CONTENT OF RESTING AND NON-RESTING LARVAE AND ITS PROBABLE SIGNIFICANCE

The foregoing investigations indicate that the moisture content of the insect's food greatly influences its future development, i.e., whether the mature larvae pupate immediately or enter the diapause. It was therefore deemed important to measure the moisture content of resting and non-resting larvae. This was accomplished by drying the larvae in an oven at 215° F. for 48 hours.

Table 6.---Moisture content of resting and non-resting pink bollworm larvae, Mayaguez, P.R., 1937.

Type of Larvae	Samples	Moisture Content		
		Average	Max.	Min.
	Number	Percent	Percent	Percent
Non-resting	1 1/	70.8		
Resting	11 1/	62.9-41.46	65.4	60.8

1/ Each sample contained 25 or more larvae.

As shown in table 6, the moisture content on non-resting larvae averaged 7.9 percent higher than resting larvae. This condition was probably caused by the difference in the moisture content of their food. These results are of special interest when compared with the results obtained by Robinson in connection with this subject. He (1/ 2/) found that a direct relationship exists between the water content of various species of insects and their food, i.e., species which live on food containing a low percentage of water have themselves a small amount of water in their own tissues, and vice versa. He further shows that some species of insects which live under low moisture conditions have the remarkable capacity of adapting themselves to such conditions by binding on the surface of their colloids a large proportion of the available water and in this way protecting themselves against loss by evaporation. These studies on the diapause in the pink bollworm indicate a similar relationship.

1/ Robinson, W. 1928. Response and adaption of insects to external stimuli. Ann. Ent. Soc. Amer. 21 (3) : 407-417.

2/ Robinson, W. 1928. Water conservation in insects. Jour. Econ. Ent. 21(6) : 897-901.

THE ENVIRONMENTAL COMPLEX AND ITS RELATION TO THE DIAPAUSE

A study of pink bollworm larvae in relation to changes in the environmental complex over a period of years permits the evaluation of the action of the environment on its behavior, i.e., how changes in the environmental complex induce the development of the diapause. The most important factor in this connection under tropical conditions is the moisture content of the bolls, the insect's food. This, of course, is dependent upon the amount of water available to the cotton plant, which in turn is dependent upon the combined effects of the following factors: (1) soil moisture, which is determined by precipitation, temperature, evaporation, air movement, nebulosity, relative humidity, etc.; (2) soil texture, which is again affected by the size of the soil particles, organic matter, number of living organisms, etc.; and (3) the number of plants per unit area in competition for moisture.

TERMINATION OF THE DIAPAUSE

In order to evaluate the importance of rainfall on the duration of the diapause, experiments were conducted in the laboratory so that the various factors could be controlled separately. Four hundred and fifty heavily infested open cotton bolls were placed in each of two cages. The bolls in one cage were kept continuously moist by frequent applications of water. The bolls in the other cage were left dry. Adult emergence from each cage was recorded daily. The results are summarized in table 7. It will be noted that moisture greatly accelerated emergence. Fifty percent of the moths had emerged from the moistened bolls 32.3 days after date of installation, and all had emerged after 54 days. From the bolls kept under dry conditions 50 percent had not emerged until 109.9 days had passed, and all had not emerged until after 258 days. The maximum duration of the diapause in the field was found to be 172 days during a period of severe drought.

These results show that moisture is the important factor limiting the duration of the diapause under tropical conditions, and that in the field, under conditions of heavy rainfall, pupation and emergence of all resting larvae would be completed in about 60 days. They also indicate that it may perhaps be possible to reduce pink bollworm damage by adjusting the planting date to the distribution of rainfall, or by irrigation.

Table 7.---Comparative rate of emergence by 20 day periods of pink bollworm moths from resting larvae in open cotton bolls when held under dry and moist conditions in the insectary. Mayaguez, P.R., 1937. /a

Date	Period in days after date of installation	Accumulated Emergence From			
		Dry Cotton Bolls		Moist Cotton Bolls	
		Number	Percent	Number	Percent
Apr. 17	0	0	0	0	0
May 7	20	0	0	9	10.3
May 27	40	0	0	69	79.3
June 16	60	11	2.6	87	100.0
July 6	80	90	21.5		
July 26	100	173	41.4		
Aug. 15	120	298	71.3		
Sept. 4	140	363	86.8		
Sept. 24	160	396	94.7		
Oct. 14	180	402	96.2		
Nov. 3	200	405	96.9		
Nov. 23	220	410	98.1		
Dec. 13	240	414	99.0		
Dec. 31	260	418	100.0		

/a Four hundred and fifty heavily infested open bolls were placed in each of two cages on April 17, each cage containing approximately 389 long-cycle larvae.

SUMMARY AND CONCLUSIONS

Over a period of years, the behavior of pink bollworm larvae has been studied in relation to definite changes in the environmental complex in order to determine the combination of factors that initiates the development and termination of the diapause in the field. In the laboratory, controlled experiments were conducted in which environmental changes were induced in order to aid in the interpretation of the effect of the environmental complex on the larval stage.

It was found that larvae undergoing the diapause occur in abundance during periods of drought, regardless of the age of the crop or the time of the year at which this takes place. A few resting larvae always occur in the field regardless of the amount of rainfall - an indication of the existence of an inherited cycle which cannot be broken within several generations.

Dry food caused by drought conditions, i.e., lack of rainfall, high temperatures, low humidity, excessive air movement, etc., reduces the water content of the larvae themselves, thereby decreasing the rate of metabolism, and causing cessation of development.

The moisture content of the seed and lint was inversely proportional to the age of the boll, the coefficient of correlation being -0.986 ± 0.005. The moisture content of the seed and lint was also inversely proportional to the percentage of resting larvae, the coefficient of correlation being -0.552 ± 0.169.

Moisture is the most important factor limiting the duration of the diapause under tropical conditions; and in the field, under conditions of heavy rainfall, pupation and emergence of all resting larvae would be completed in about 60 days.

These studies suggest that it may be possible to reduce pink bollworm damage, since the abundance of the diapause in the field, and therefore the carry-over, may be partially controlled by adjusting the planting date to the distribution of rainfall, or by irrigation.

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(Read at the meeting of the American Association of Economic Entomologists, February 21, 1939, Tampa, Florida)